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Dated 6 October 1999.

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11SEP98 E389796-1 000754
P01/7700 25.00 - 9819882.3

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference M2579

2. Patent application number
(The Patent Office will fill in this part) 11 SEP 1998 **9819882.3**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

TISSUE SCIENCE LABORATORIES LIMITED, Greyholme House, 49 Victoria Road, Aldershot, Hants GU11 1SJ

Patents ADP number *(if you know it)* 7511074001

If the applicant is a corporate body, give the country/state of its incorporation England

4. Title of the invention

INJECTABLE COLLAGENOUS TISSUE COMPOSITIONS

5. Name of your agent *(if you have one)* GALLAFENT & CO

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* 9 STAPLE INN
LONDON WC1V 7QH

Patents ADP number *(if you know it)* 0000729001

6. If you are declaring priority from one or more earlier patent applications give the country and the date of filing of the or of each of these earlier applications and *(if you know it) the or each application number*

Country	Priority application number *(if you know it)*	Date of filing *(day/month/year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day/month/year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
(See note (d))

Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

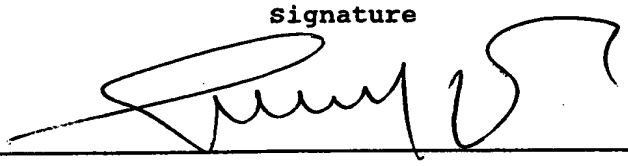
Continuation sheets of this form	
Description	6
Claim(s)	-
Abstract	-
Drawings	-

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translation of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination and search *(Patents Form 10/77)*	
Any other documents *(Please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature



Date

11 September 1998

12. Name and daytime telephone number of person to contact in the United Kingdom

GALLAFENT & CO
0171 242 3094

INJECTABLE COLLAGENOUS TISSUE COMPOSITIONS

This invention relates to injectable collagenous tissue compositions.

In recent years, much attention has been given to the development of compositions and preparations for wound treatment and for use in general and plastic surgery, in particular for the improved restoration of surgically induced wounds or for the correction of physiological malfunction as, for example, of the urethral sphincter in cases of urinary incontinence.

Much attention has been focussed on the provision of materials based on collagen, either of human or animal origin. In particular, considerable attention has been directed to developing preparations and materials based on animal tissues which are treated to provide compatibility, i.e. to avoid rejection of the tissues when used on humans.

Earlier work by the inventors of the present application is reflected in United States Patent Specification 5397353 and EP-A-182842 which disclose methods of

preparing collagenous materials, preferably in sheet form, and which are suitable for transplantation. The treatment is designed to produce a collagenous material which is non-antigenic so that it is not rejected and which is non-resorbable so that it forms a permanent transplant. In particular, the material described in these specifications retains the natural structure and original architecture of the natural tissue; the molecular ultrastructure of the collagen is retained. These materials have proved highly satisfactory in practice and, in particular, have shown themselves to be capable of being re-vascularised once implanted while, at the same time, being resistant to calcification. They are particularly useful in hernia repair and plastic surgery for the elevation of depressed scars.

The compositions described in United States Patent Specification 5397353, however, are disclosed as large scale structures, for example 0.255 mm thick and usually presented as sheets varying in size from 1 cm^2 to 12 cm^2. This is useful for specific implant use, e.g. during restorative surgery, but is unsuited for use generally to build up soft tissues.

In cosmetic and reconstructive surgery, e.g. for the repair of small acne scars and for elevating and smoothing wrinkles, it is often desirable to use material for tissue implantation or so-called augmentation which can be injected into the desired site.

Various so-called injectable implant materials have been developed for such purposes. United States Patent Specifications 5523291, 5676698 and 5705488 disclose injectable implant compositions for soft tissue augmentation comprising elastin and collagen and a

biocompatible carrier, or flexible pouches containing such a material. The difficulty with such materials as are disclosed in these United States specifications, however, is that there is a tendency to resorption and this can mean that the implant is effective only for a limited time. Additionally, such materials do not encourage vascularisation, i.e. they do not integrate well into the surrounding healthy tissue following implantation.

We have now surprisingly found that the favourable properties including resistance to resorption, resistance to calcification, and the ability to become recellularized and revascularised, which characterise the large scale structures disclosed in Specification 5397353 are capable of being retained consistently with the presentation of the implant material as an injectable composition.

According to a first feature of the present invention, there is accordingly provided an injectable implant composition which comprises a biocompatible carrier medium having dispersed therein particles of collagenous material where the particles are sufficiently small to enable the composition to be injected, but sufficiently large to preserve the original architecture and molecular structure of the natural tissue material from which they are derived, and wherein the collagenous material is substantially free of non-fibrous tissue proteins, glycoproteins, cellular elements and lipids or lipid residues, and which is non-cytotoxic. Preferably, the material is free or substantially free of antigenic polysaccharides and mucopolysaccharides. The biocompatible medium may be, for example, a saline or dextran solution.

Such materials may be prepared from collagenous materials of human or animal origin, the preferred starting material being pig dermis, by methods as disclosed in Specification 5397353 or analogously thereto. Depending on the starting material, the composition may contain a proportion of elastin. It is then possible, provided care is taken, to reduce the material from large pieces to small particles which can then be formulated into a sterile injectable composition. Care must however be taken to ensure that such size reduction is not accompanied by degradation of the molecular structure of the original material. The preferred method of providing particles of an appropriate size is by grinding or milling and this is preferably carried out in a ball or hammer mill which may be cooled to an appropriate temperature.

The collagenous material may be, if desired, crosslinked, e.g. using a diisocyanate, in order to make it resistant to collagenolytic enzymes and thus render it substantially non-resorbable.

The preferred method of rendering the compositions sterile is by gamma irradiation.

The preferred particle size of the particles of collagenous material in the injectable compositions according to the present invention is from 50 to 500 microns. The particle size distribution may vary but preferably at least 50% of the particles are within ± 25% of the average particle size. The concentration of solids in the injectable composition is preferably in the range of 10 to 70% (v/v).

The following examples will serve to illustrate the invention:

Example 1

Under sterile conditions, samples of porcine dermal collagen were cut into small pieces (1 to 3 mm^3) and dehydrated using several changes of 100% ethanol and anhydrous acetone. Using a ball mill, the dried collagen pieces were ground and sieved to produce a fine white powder. The sieved powdered collagen was rehydrated in sterile phosphate buffered saline to produce a collagen suspension concentration of 60 to 70% (v/v).

Example 2

Small pieces of wet porcine dermal collagen were frozen in liquid nitrogen and ground in a freezer mill. The ground collagen fragments were suspended in sterile phosphate buffered saline to produce a collagen suspension concentration of 60 to 70% (v/v).

Example 3

To directly examine cell/collagen biointeraction, sieved powdered porcine dermal collagen was rehydrated in complete mammalian cell culture medium to produce a collagen suspension concentration of 70% (v/v) and seeded with either primary human foreskin fibroblasts or primary rat skin dermal fibroblasts. Collagen/fibroblast samples were aliquoted into costar wells and incubated at 37°C, 5 to 7% (v/v) CO_2 saturated humidity. As studied over a 21 day incubation period, both human and rat fibroblasts proliferated and migrated into and adhered to the porcine collagen fragments which they assembled into densely packed clumps with clear evidence of de novo collagen synthesis.

Example 4

To examine in vivo performance collagen suspensions were injected (0.2 ml/injection) intracutaneously into dorsal sites in isogenic PVG/Ola rats. Sequential biopsies up to 8½ month post injection showed the persisting macroscopic presence of injected collagen as subdermally located white discs with no overt signs of loss of injected collagen mass nor of adverse host reactions.